

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Yuan-Tsong Chen, et al.                      Art Unit : 1634  
Serial No. : 10/705,245                                      Examiner : KAPUSHOC, STEPHEN  
Filed : November 10, 2003                                      THOMAS

Title : RISK ASSESSMENT FOR ADVERSE DRUG REACTIONS

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION OF UNDER 37 C.F.R. § 1.132**

I, Yuan-Tsong Chen, declare:

1. I am a co-inventor named in the above-captioned U.S. patent application.

2. I understand that an Office Action dated September 7, 2007 is outstanding in the present application. Amended claim 1, an independent claim under examination, covers a method of assessing the risk of a human patient for developing carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) based on the presence of HLA-B\*1502 in that patient.

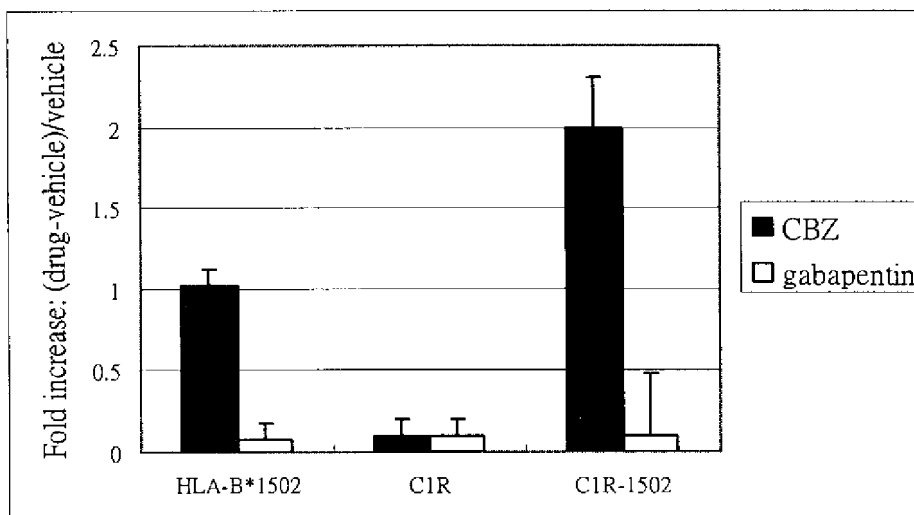
3. SJS and TEN are severe cutaneous hypersensitivity reactions caused by drugs; e.g., CBZ. It is believed that both CBZ-induced SJS and TEN are mediated by CBZ-activated cytotoxic T cells, which initiate apoptosis of keratinocytes resulting in erosions of skin and mucous membranes. See Roujeau et al., *New England J. Med.*, 331:1272-1285, 1994 (copy attached as Exhibit A). CBZ and its metabolites have been found to activate T cells isolated from hypersensitivity patients via direct interaction with MHC and specific T-cell receptors. See Wu et al., *J. Allergy Clin. Immunol.*, 118:233-241, 2006 (copy attached as Exhibit B).

4. I, or others under my supervision, have conducted experiments to demonstrate that HLA-B\*1502, an MHC class I allele, is directly involved in activating CBZ-specific cytotoxic T cells, the effector cells in SJS/TEN (causing cell death against cells expressing HLA-B\*1502).

We first examined whether activation of CBZ-specific T cells is HLA-B\*1502 restricted. Peripheral blood mononuclear cells (PBMCs) were isolated from SJS/TEN patients carrying HLA-B\*1502. CBZ-specific T cells contained in the isolated PBMCs were then enriched as follows.  $1 \times 10^5$  PBMCs, prepared by Ficoll-Isopaque density gradient centrifugation, were

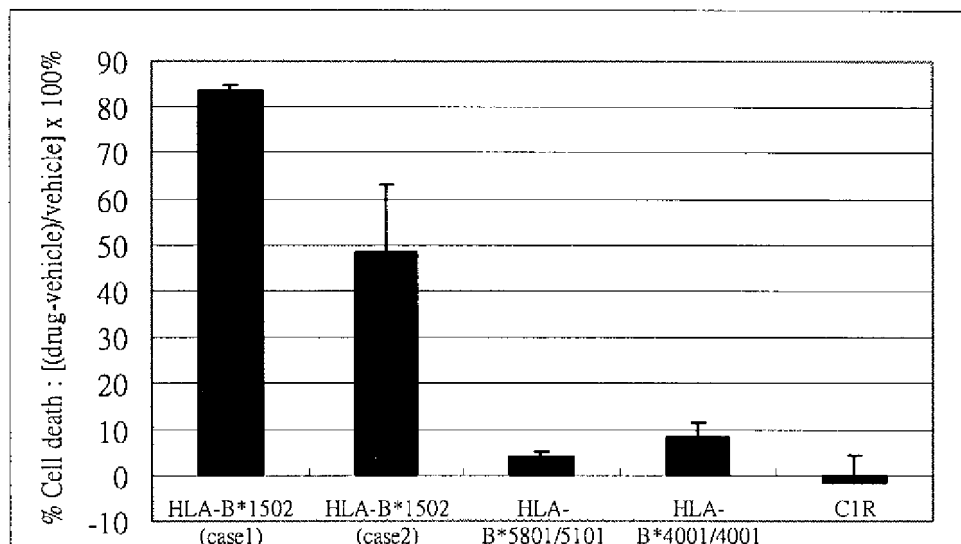
cultured in complete RPMI medium containing 10% heat-inactivated human serum, IL-2 (25U/ml), and CBZ (25 $\mu$ g/ml) in a 37 °C, 5% CO<sub>2</sub> incubator for 7 days to proliferate CBZ-specific T cells. The proliferated T cells were further expanded by co-culturing with autologous B cells in the presence of CBZ for 7 days. This co-culturing process was repeated three times and the CBZ-specific T cells thus enriched were subjected to determination of their HLA restriction. Briefly, in the presence of a control vehicle, CBZ, or gabapentin, the T cells ( $5 \times 10^3$ ) were co-cultured with one of the following three types of antigen-presenting cells: (1) Epstein-Barr virus (EBV)-transformed B cells that carry HLA-B\*1502 (B-1502), (2) C1R cells (CRL-1993, American Type Culture Collection; HLA-A and HLA-B deficient), and (3) HLA-B\*1502-transfected C1R cells (C1R-1502). Note that both CBZ and gabapentin are anticonvulsants. After incubated for 24 hours, the T cells were harvested and subjected to ELISPOT analysis to determine the number of IFN- $\gamma$  positive T cells, which are activated T cells. As shown in Figure 1 below, CBZ activated T cells when B-1502 and C1R-1502, but not C1R, were used as antigen presenting cells. Gabapentin failed to activate T cells, regardless of which types of antigen presenting cells were used. These results clearly indicate that CBZ activates T cells via presentation by HLA-B\*1502.

**Figure 1**



Next, we examined the cytotoxicity of CBZ-specific T cells against B cells expressing HLA-B\*1502 or other HLA-B alleles. The enriched CBZ-specific T cells described above were co-cultured with four types of B cells: (1) EBV-transformed B cells established from two HLA-B\*1502 carriers (case 1 and case 2), (2) EBV-transformed B cells that carry HLA-B\*5801/5101 (B-5801/5101), (3) EBV-transformed B cells that carry HLA-B\*4001/4001 (B-4001/4001), and (4) C1R, in the presence of CBZ or the vehicle control. After 4-hour incubation, cells were collected and subjected to flow cytometry to determine the death rates of the B cells. As shown in Figure 2 below, the death rates of the B cells expressing HLA-B\*1502 (case 1 and case2) are much higher than those of B-5801/5101, B-4001/4001, and C1R. This data demonstrates that the CBZ-activated T cells are cytotoxic against antigen-presenting cells expressing HLA-B\*1502 specifically.

**Figure 2**



In sum, the experimental data presented above indicate that CBZ activates T cells via presentation by HLA-B\*1502 and that the activated T cells show strong cytotoxicity against cells expressing HLA-B\*1502. In other words, HLA-B\*1502 is a critical player in activating cytotoxic CBZ-specific T cells, which are the effector cells in CBZ-induced SJS/TEN. Clearly, HLA-B\*1502 is directly involved in the development of SJS/TEN. It follows that a HLA-

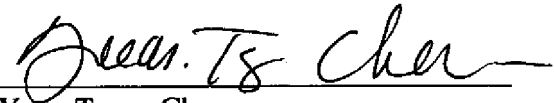
Applicant : Yuan-Tsong Chen, et al  
Serial No. : 10/705,245  
Filed : November 10, 2003  
Page : 4 of 4

Attorney's Docket No.: 70003-003001/ 12A-920716

B\*1502 carrier, regardless of his or her ethnic background, is at risk for developing SJS/TEN.

5. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: Dec 5, 2007

  
Yuan-Tsong Chen  
Director  
Institute of Biomedical Sciences  
Academia Sinica  
Taipei, Taiwan

Professor of Pediatrics and Genetics  
Duke University Medical Center  
Durham, NC, USA